

PATENT COOPERATION TREATY  
PCT  
INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 88346	<b>FOR FURTHER ACTION.</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416).	
International Application No. PCT/AU00/00021	International Filing Date (day/month/year) 14 January 2000	Priority Date (day/month/year) 14 January 1999
International Patent Classification (IPC) or national classification and IPC Int. Cl. <sup>7</sup> A23L 1/0522; A61K 35/72, 35/74, 47/36; C12N 11/10		
Applicant FOOD TECHNOLOGY INNOVATIONS PTY LIMITED et al		

1.	This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.																
2.	This REPORT consists of a total of 4 sheets, including this cover sheet. <input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT). These annexes consist of a total of 10 sheet(s).																
3.	This report contains indications relating to the following items: <table border="0"><tr><td>I</td><td><input checked="" type="checkbox"/> Basis of the report</td></tr><tr><td>II</td><td><input type="checkbox"/> Priority</td></tr><tr><td>III</td><td><input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</td></tr><tr><td>IV</td><td><input type="checkbox"/> Lack of unity of invention</td></tr><tr><td>V</td><td><input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</td></tr><tr><td>VI</td><td><input checked="" type="checkbox"/> Certain documents cited</td></tr><tr><td>VII</td><td><input type="checkbox"/> Certain defects in the international application</td></tr><tr><td>VIII</td><td><input type="checkbox"/> Certain observations on the international application</td></tr></table>	I	<input checked="" type="checkbox"/> Basis of the report	II	<input type="checkbox"/> Priority	III	<input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability	IV	<input type="checkbox"/> Lack of unity of invention	V	<input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement	VI	<input checked="" type="checkbox"/> Certain documents cited	VII	<input type="checkbox"/> Certain defects in the international application	VIII	<input type="checkbox"/> Certain observations on the international application
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Date of submission of the demand 19 June 2000	Date of completion of the report 9 March 2001
Name and mailing address of the IPEA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA E-mail address: pct@ipaaustralia.gov.au facsimile No. (02) 6285 3929	Authorized Officer  CHRISTOPHER LUTON Telephone No. (02) 6283 2256

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/AU00/00021

## I. Basis of the report

1. With regard to the elements of the international application:\*

☐ the international application as originally filed.☒ the description, pages 1, 2, 6-21, as originally filed,

pages , filed with the demand,

pages 3, 4, 5, received on 8 November 2000 with the letter of 8 November 2000

☒ the claims, pages , as originally filed,

pages , as amended (together with any statement) under Article 19,

pages , filed with the demand,

pages 22-28, received on 1 March 2001 with the letter of 28 February 2001

☒ the drawings, pages 1-14, as originally filed,

pages , filed with the demand,

pages , received on with the letter of

☐ the sequence listing part of the description:

pages , as originally filed

pages , filed with the demand

pages , received on with the letter of

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item. These elements were available or furnished to this Authority in the following language which is:

☐ the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).☐ the language of publication of the international application (under Rule 48.3(b)).☐ the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, was on the basis of the sequence listing:

☐ contained in the international application in written form.☐ filed together with the international application in computer readable form.☐ furnished subsequently to this Authority in written form.☐ furnished subsequently to this Authority in computer readable form.☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished4. ☐ The amendments have resulted in the cancellation of:☐ the description, pages☐ the claims. Nos.☐ the drawings, sheets/fig.5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).\*\*

\* Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).

\*\* Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

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## V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

## 1. Statement

Novelty (N)	Claims 1-75	YES
	Claims	NO
Inventive step (IS)	Claims 1-75	YES
	Claims	NO
Industrial applicability (IA)	Claims 1-75	YES
	Claims	NO

## 2. Citations and explanations (Rule 70.7)

The following documents identified in the International Search Report have been considered for the purposes of this report:

D1 - WO 97/34591

D2 - WO 97/34615

D3 - WO 97/34592

D4 - WO 96/08261

D5 - Brown et al.

The citations teach preparations comprising microorganisms together with resistant starch. The citations teach that growth and survival of microbes provided in such compositions is enhanced. The citations teach that resistant starch is useful as both a carrier for microbes and also as a growth and maintenance medium. The citations teach that resistant starch improves the robustness of probiotic cultures and improves the viability of probiotic strains in processed foods.

However, the citations do not teach that microbes grown on resistant starch and subsequently harvested (viz. removed) therefrom, demonstrate improved viability during future use. Hence, the claims are considered to be novel and to involve an inventive step in light of the citations.

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## VI. Certain documents cited

## 1. Certain published documents (Rule 70.10)

Application No. Patent No.	Publication date (day/month/year)	Filing date (day/month/year)	Priority date ( valid claim) (day/month/year)
WO 99/04649	4 February 1999	16 July 1998	25 July 1997

## 2. Non-written disclosures (Rule 70.9)

Kind of non-written disclosure	Date of non-written disclosure (day/month/year)	Date of written disclosure referring to non- written disclosure (day/month/year)
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addition of the resistant starch to the microbial preparations after growth of the microbe also further enhances robustness of the microbes, thus leading to enhanced survival of the microorganisms. The discovery is also applicable to biocontrol and bioremediation preparations as well as food fermented by the addition of microorganisms, starter cultures, since more robust starter cultures can also be produced by growth on resistant starch medium and/or addition of resistant starch to subsequent products as described above.

#### Disclosure of the Invention

In a first general aspect, the present invention provides an improved microbial preparation including microbes having an increased survival/recovery rate in a product or fermentation. Preferably, the product is a food, feed, nutraceutical, pharmaceutical, biocontrol or bioremediation product. Preferably, the microbial preparation comprises microbes previously grown in media based on, or containing, resistant starch such that the harvested microbes have an increased survival/recovery rate in subsequent use. One form of resistant starch particularly suitable for the present invention is starch containing resistant starch, particularly high amylose maize starches or materials derived from high amylose maize starches. In subsequent use, the preparation may be mixed with additional resistant starch to further enhance the growth/yield potential, or increased survival/recovery rate of the microbes in the product. It has been found that growth in media based on, or containing, soluble starch, maltose or resistant starch seems to enhance the microbes ability to bind to additional resistant starch added to the product therefore enhancing the growth/yield potential, or increased survival/recovery rate of the microbes.

The microbes in the improved microbial preparation according to the first aspect of the present invention are particularly unaffected by stresses including aeration, sheer, freeze drying, freezing, drying including high, medium and low water activity, elevated temperatures, low temperatures, pressure and pressure fluctuations, low pH, high pH, bile acids, moisture, high or low osmolarity, high salt, or combinations thereof. The microbial preparation according to the present invention is particularly suitable for use in probiotics, starter cultures, biocontrol or bioremediation agents.

In a second aspect, the present invention provides a process of preparing a microbial preparation having an increased survival/recovery rate in a product, the process comprising growing or culturing microbes in media

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based on or containing resistant starch such that the cultured microbes will have an increased survival/recovery rate when used subsequently in a product, and harvesting the cultured microbes having an increased survival/recovery rate, wherein in subsequent use in a product, the microbes  
5 have an increased survival/recovery rate in the product compared with the same microbes grown or cultured in media without resistant starch and used in a similar product.

In a third aspect, the present invention provides an improved microbial preparation having an increased survival/recovery rate in a product prepared  
10 by the process according to the second aspect of the present invention.

In a fourth aspect, the present invention provides a product containing microbes having an increased survival/recovery rate, the product including a microbial preparation according to the first or third aspects of the present invention.

15 Preferably, the product is a food, feed, nutraceutical, pharmaceutical, biocontrol or bioremediation product. In one preferred embodiment, the food product also includes resistant starch so as to further enhance growth/yield potential, or survival/recovery rate of the microbes.

In a fifth aspect, the present invention relates to the use of resistant  
20 starch in microbial culture media to produce microbes having an increased survival/recovery rate in a product compared with the same microbes grown or cultured in media without resistant starch.

One form of resistant starch particularly suitable for the present invention is starch containing resistant starch. Preferably, the starches have  
25 an amylose content of at least 40% (w/w). In a preferred form the starch is from maize having an amylose content of at least 70% (w/w), at least 80% (w/w) or at least 90% (w/w). The starch can also be chemically, physically, or enzymically treated or modified. Chemical modification can be by oxidation, cross-bonding, etherification, esterification, acidification,  
30 dextrinisation, or mixtures thereof. Starches can also be treated to enhance the resistant starch content by a number of physical or chemical means. One preferred means is to heat-treat starch in the presence of moisture (heat-moisture treatment) which can be achieved by a number of processes including heating under negative, atmospheric or positive pressure under  
35 elevated moisture, or cycling techniques through different temperatures and pressures. Heating can be in the order of 100 to 180°C, preferably around 120

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to 150°C and moisture levels of 10 to 80%, preferably 20 to 60%. Repeated autoclaving and rapid cooling can also be used to increase the resistant starch content of starches. It will be appreciated that these processes and conditions can be changed to achieve the desired increase in the level of resistant starch in the starch being treated. Treatment can also be by solvent extraction to remove fats and/or minerals from the starch.

There are a variety of probiotic microorganisms which are suitable for use in this invention including yeasts such as *Saccharomyces*, and bacteria such as the genera *Bifidobacterium*, *Bacteroides*, *Clostridium*, *Fusobacterium*, *Propionibacterium*, *Streptococcus*, *Enterococcus*, *Lactococcus*, *Staphylococcus*, *Leuconostoc*, *Peptostreptococcus* and *Lactobacillus*. The invention is not, however, limited to these particular microorganisms. Preferably, the starter cultures include, but not limited, to lactic acid bacteria including lactobacillus, lactococcus and streptococcus, leuconostoc, and yeasts. Preferably, the microorganisms for use in biocontrol or bioremediation products include bifidobacteria, acidophilus, fungi, *Bacillus* species, pseudomonads and Alcaligenes. It will be appreciated, however, that other species of microorganisms would also be suitable candidates for use according to the present invention.

The invention also includes microorganisms of different strains or species, including non-starch utilisers, to interact and demonstrate improved growth and/or activity in the large bowel, nasal tract or vaginal tract.

In a preferred embodiment of the first, second, third and fourth aspects of the present invention, the microbial preparations are starter cultures or probiotic preparations which can be liquid, frozen or dried. The preparations may also include food and feed products containing other microbial additives. These products include fluid-based or solid-based products. Fluid-based food products include milk-based products where the edible ingredient is one or more milk-based ingredients including whole milk, milk solids, milk fat, cream, non-fat dried milk, any other component or derivative from milk that can be used in milk-based products, water-based fluids, cereal and plant extracts such as soy-based beverages and additives. Solid-based food products include snack bars, breakfast cereals, bread, confectionary, extruded food products, muesli bars, buns, biscuits, feed pellets, coated food products, tablets, food additives, health supplements, and pharmaceutical preparations.

## CLAIMS:

1. A microbial preparation comprising harvested microbes which have been grown or cultured in a media based on or containing resistant starch in a manner such that when subsequently incorporated in a product, the  
5 survival/recovery rate of the harvested microbes is increased as compared with the same microbes grown or cultured in a media without resistant starch.
2. The microbial preparation according to claim 1 wherein the product is selected from the group consisting of a food, feed, nutraceutical,  
10 pharmaceutical, biocontrol, and bioremediation product.
3. The microbial preparation according to claim 1 or 2 further including resistant starch.
4. The microbial preparation according to any one of claims 1 to 3 wherein the resistant starch is type RS1, RS2, RS3 or RS4.
- 15 5. The microbial preparation according to claim 4 wherein the resistant starch is derived from starch selected from the group consisting of maize, rice, barley, wheat, legumes, potatoes, and bananas.
6. The microbial preparation according to claim 5 wherein the resistant starch is derived from a starch having an amylose content of at least 40%  
20 (w/w).
7. The microbial preparation according to claim 6 wherein the resistant starch is derived from maize starch.
8. The microbial preparation according to claim 7 wherein the maize starch having an amylose content of at least 70% (w/w).
- 25 9. The microbial preparation according to claim 7 wherein the maize starch having an amylose content of at least 80% (w/w).
10. The microbial preparation according to claim 7 wherein the maize starch having an amylose content of at least 90% (w/w).
11. The microbial preparation according to any one of claims 5 to 10  
30 wherein the starch is chemically, physically, and/or enzymically treated or modified.
12. The microbial preparation according to claim 11 wherein the chemical modification is selected from the group consisting of oxidation, cross-bonding, etherification, esterification, acidification, dextrinisation, and  
35 mixtures thereof.



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13. The microbial preparation according to claim 11 wherein the physical treatment is heat-moisture treatment to enhance or increase the resistant starch content of the starch.
14. The microbial preparation according to claim 11 wherein the treatment is by solvent extraction to remove fats and/or minerals from the starch.
15. The microbial preparation according to any one of claims 1 to 14 wherein in use the microbes are substantially unaffected by stresses including aeration, sheer, freeze drying, freezing, drying including high, medium and low water activity, elevated temperatures, low temperatures, pressure and pressure fluctuations, low pH, high pH, bile acids, moisture, high osmolality, low osmolality, high salt, or combinations thereof.
16. The microbial preparation according to any one of claims 1 to 15 being a probiotic, a starter culture, a biocontrol or bioremediation product.
17. The microbial preparation according to claim 16 wherein the microbes are probiotic microorganisms from the genera selected from the group of consisting of *Saccharomyces*, *Bifidobacterium*, *Bacteroides*, *Clostridium*, *Fusobacterium*, *Propionibacterium*, *Streptococcus*, *Enterococcus*, *Lactococcus*, *Staphylococcus*, *Peptostreptococcus*, and *Lactobacillus*.
18. The microbial preparation according to claim 16 wherein the microbes are starter cultures selected from the group consisting of lactic acid bacteria including lactobacillus, lactococcus and streptococcus, leuconostoc, and yeasts.
19. The microbial preparation according to claim 16 wherein the microbes are suitable for use in biocontrol or bioremediation being selected from the group consisting of bifidobacteria, acidophilus, fungi, *Bacillus* species, pseudomonads and *Alcaligenes*.
20. A process of preparing a microbial preparation having an increased survival/recovery rate in a product, the process comprising growing or culturing microbes in a media based on or containing resistant starch in a manner such that when subsequently incorporated in a product the survival/recovery rate of the harvested microbes is increased as compared with the same microbes grown or cultured in a media without resistant starch, and harvesting the cultured microbes having an increased survival/recovery rate.

21. The process according to claim 20 wherein the product is selected from the group consisting of a food, feed, nutraceutical, pharmaceutical, biocontrol, and bioremediation product.
22. The process according to claim 20 or 21 wherein the resistant starch is  
5 type RS1, RS2, RS3 or RS4.
23. The process according to claim 22 wherein the resistant starch is derived from starch selected from the group consisting of maize, rice, barley, wheat, legumes, potatoes, and bananas.
24. The process according to claim 23 wherein the resistant starch is  
10 derived from a starch having an amylose content of at least 40% (w/w).
25. The process according to claim 24 wherein the resistant starch is derived from maize starch.
26. The process according to claim 25 wherein the maize starch having an amylose content of at least 70% (w/w).
- 15 27. The process according to claim 25 wherein the maize starch having an amylose content of at least 80% (w/w).
28. The process according to claim 25 wherein the maize starch having an amylose content of at least 90% (w/w).
29. The process according to any one of claims 23 to 28 wherein the starch  
20 is chemically, physically, and/or enzymically treated or modified.
30. The process according to claim 29 wherein the chemical modification is selected from the group consisting of oxidation, cross-bonding, etherification, esterification, acidification, dextrinisation, and mixtures thereof.
- 25 31. The process according to claim 29 wherein the physical treatment is heat-moisture treatment to enhance or increase the resistant starch content of the starch.
32. The process according to claim 29 wherein the treatment is by solvent extraction to remove fats and/or minerals from the starch.
- 30 33. The process according to any one of claims 20 to 32 wherein the resistant starch is used in the media at a concentration of 0.01 to 10% (w/w).
34. The process according to claim 33 wherein the resistant starch is used in the media at 0.1 to 5% (w/w).
- 35 35. The process according to claim 33 wherein the resistant starch is used in the media at 1% (w/w).

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36. The process according to any one of claims 20 to 35 wherein in use the microbes are unaffected by stresses including aeration, sheer, freeze drying, freezing, drying including high, medium and low water activity, elevated temperatures, low temperatures, pressure and pressure fluctuations, low pH, high pH, bile acids, moisture, high osmolarity, low osmolarity, high salt, or combinations thereof.
37. The process according to any one of claims 20 to 36 wherein the microbial preparation is a probiotic, a starter culture, a biocontrol or bioremediation product.
38. The process according to claim 37 wherein the microbes are probiotic microorganisms from the genera selected from the group of consisting of *Saccharomyces*, *Bifidobacterium*, *Bacteroides*, *Clostridium*, *Fusobacterium*, *Propionibacterium*, *Streptococcus*, *Enterococcus*, *Lactococcus*, *Staphylococcus*, *Peptostreptococcus*, and *Lactobacillus*.
39. The process according to claim 37 wherein the microbes are starter cultures selected from the group consisting of lactic acid bacteria lactic acid bacteria including lactobacillus, lactococcus and streptococcus, leuconostoc, and yeasts.
40. The process according to claim 37 wherein the microbes are suitable for use in biocontrol or bioremediation being selected from the group consisting of bifidobacteria, acidophilus, fungi, *Bacillus* species, pseudomonads and *Alcaligenes*.
41. A microbial preparation having an increased survival/recovery rate in a product prepared by the process according to any one of claims 20 to 40.
42. A product containing microbes having an increased survival/recovery rate, the product including a microbial preparation according to any one of claims 1 to 19 or 41.
43. The product according to claim 42 selected from the group consisting of a food, feed, nutraceutical, pharmaceutical, biocontrol, and bioremediation product.
44. The product according to claim 43 being a food, feed, nutraceutical or pharmaceutical product selected from the group consisting of fluid-based food products, water-based fluids, cereal and plant-based food products, solid-based food products, tablets, food additives, health supplements, and pharmaceutical preparations.

45. The product according to claim 44 wherein the fluid-based food products include milk-based products where the edible ingredient is one or more milk-based ingredients including whole milk, milk solids, milk fat, cream, non-fat dried milk, any other component or derivative from milk
- 5 suitable for use in milk-based products.
46. The product according to claim 44 wherein the solid-based food products include snack bars, breakfast cereals, bread, confectionary, extruded food products, muesli bars, buns, biscuits, feed pellets, and coated food products.
- 10 47. The product according to claim 43 being a food product suitable to contain and deliver probiotic microorganisms.
48. The food product according to claim 47 selected from the group consisting of food stuffs, fruit beverages, water ices, confectionary, coatings or covertures, yoghurts, yoghurt drinks, unfermented drinks, flavoured milk
- 15 drinks, modified milk drinks, ice-creams, and dairy desserts.
49. The product according to any one of claims 42 to 48 further including resistant starch.
50. The product according to claim 49 wherein the resistant starch is type RS1, RS2, RS3 or RS4.
- 20 51. The product according to claim 50 wherein the resistant starch is derived from starch selected from the group consisting of maize, rice, barley, wheat, legumes, potatoes, and bananas.
52. The product according to claim 51 wherein the resistant starch is derived from a starch having an amylose content of at least 40% (w/w).
- 25 53. The product according to claim 51 wherein the resistant starch is derived from maize starch.
54. The product according to claim 53 wherein the maize starch having an amylose content of at least 70% (w/w).
55. The product according to claim 53 wherein the high amylose starch is
- 30 maize starch having an amylose content of at least 80% (w/w).
56. The product according to claim 53 wherein the high amylose starch is maize starch having an amylose content of at least 90% (w/w).
57. The product according to any one of claims 51 to 56 wherein the starch is chemically, physically, and/or enzymically treated or modified.
- 35 58. The product according to claim 57 wherein the chemical modification is selected from the group consisting of oxidation, cross-bonding,

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etherification, esterification, acidification, dextrinisation, and mixtures thereof.

59. The product according to claim 57 wherein the physical treatment is heat-moisture treatment to enhance or increase the resistant starch content of the starch.
60. The product according to claim 57 wherein the treatment is by solvent extraction to remove fats and/or minerals from the starch.
61. The product according to any one of claims 49 to 60 wherein the resistant starch is added at a concentration of 0.1 to 90% (w/w) total product.
62. The product according to claim 61 wherein the resistant starch is used at 1 to 10% (w/w).
63. Use of resistant starch in a microbial culture media to produce microbes which when used subsequently in a product after being harvested from the media, have an increased survival/recovery rate as compared with the same microbes grown or cultured in a media without resistant starch.
64. The use according to claim 63 wherein the product is selected from the group consisting of a food, feed, nutraceutical, pharmaceutical, biocontrol, and bioremediation product.
65. The use according to claim 64 wherein the resistant starch is type RS1, RS2, RS3 or RS4.
66. The use according to claim 65 wherein the resistant starch is derived from starch selected from the group consisting of maize, rice, barley, wheat, legumes, potatoes, and bananas.
67. The use according to claim 66 wherein the resistant starch is derived from a starch having an amylose content of at least 40% (w/w).
68. The use according to claim 67 wherein the resistant starch is derived from maize starch.
69. The use according to claim 68 wherein the maize starch having an amylose content of at least 70% (w/w).
70. The use according to claim 68 wherein the maize starch having an amylose content of at least 80% (w/w).
71. The use according to claim 68 wherein the maize starch having an amylose content of at least 90% (w/w).
72. The use according to any one of claims 66 to 71 wherein the starch is chemically, physically, and/or enzymically treated or modified.

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73. The use according to claim 72 wherein the chemical modification is selected from the group consisting of oxidation, cross-bonding, etherification, esterification, acidification, dextrinisation, and mixtures thereof.
74. The use according to claim 72 wherein the physical treatment is heat-moisture treatment to enhance or increase the resistant starch content of the starch.
- 5 75. The use according to claim 72 wherein the treatment is by solvent extraction to remove fats and/or minerals from the starch.

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